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Contraceptive Effect of Sperm-Agglutinating Monoclonal Antibodies in Rabbits[1]

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ABSTRACT

Immune infertility in humans correlates clinically with the presence of anti-sperm antibodies that trap (agglutinate) sperm in semen and cervical mucus. To test whether sperm-agglutinating antibodies can be effective contraceptive agents, several mouse anti-rabbit sperm (MARS) sperm-agglutinating monoclonal antibodies (mAbs) were developed that rapidly and completely agglutinate sperm: MARS-M3 (IgM), MARS-G16 (IgG_3), and MARS-G17 (IgG_3). Contraceptive efficacy of these mAbs was tested by mixing the mAb with 0.1 ml semen (~1/5 of a whole ejaculate) immediately before artificially inseminating rabbits paracervically. This paracervical dose of semen provided a rigorous test since it delivered several thousand times more fertilizing doses than does a human ejaculate. All of the mAbs were contraceptively effective, and MARS-G16 reduced the number of fetuses per animal by 88% and 95% with doses of 150 μg and 2 mg, respectively. The contraceptive efficacy of the MARS mAbs in the rabbit suggests that human sperm-agglutinating mAbs may be effective agents for vaginal contraception in humans.

INTRODUCTION

Epidemics of unwanted pregnancy and sexually transmitted diseases (STDs), including acquired immunodeficiency syndrome (AIDS), emphasize the need for alternative "prophylactic contraceptives" for women, contraceptives that prevent STD infections as well as pregnancy. Monoclonal antibodies (mAbs) against sperm and STD pathogens, selected for specificity, potency, and safety, might be used to create improved vaginal barrier methods [1]. Although passive immunizations with antisera have been used effectively for more than a century, topical passive immunization has been little explored. However, recent advances in mAb technology, including inexpensive production of human mAbs in plants ("plantibodies") [2, 3], together with improved polymer-based methods for sustained vaginal delivery [4, 5], increase the feasibility of using human mAbs as safe and pleasing agents for passively immunizing the vagina against sperm and STD pathogens.

Clinical evidence [6–9] suggests that the presence of anti-sperm antibodies in either semen or cervical secretions can cause infertility by trapping sperm in these mucus secretions by agglutinating sperm [8, 9] or by stopping the forward progress of individual sperm through cervical mucus (the "shaking effect") [10, 11]. This suggests that selecting an mAb for its ability to agglutinate sperm, regardless of whether it can directly block sperm-egg interactions [12–15], might provide an additional approach to vaginal contraception.

Although sperm-agglutinating antibodies in semen correlate clinically with reduced fertility, prior research has not directly tested whether sperm-agglutinating antibodies can reduce fertility. In this investigation, we developed a large panel of mouse mAbs against rabbit sperm (MARS) that rapidly agglutinate rabbit sperm. From this panel, three mAbs were chosen to test for contraceptive effect when delivered vaginally in semen; MARS-M3 [16], MARS-G16 [17], and MARS-G17 were selected for the completeness and rapidity with which each agglutinates rabbit sperm. These mAbs were also characterized by indirect immunofluorescence, agglutination, guinea pig and rabbit complement-mediated sperm immobilization, and Western blots. In addition, HS-63 [18], a mouse anti-human sperm mAb that binds to but does not agglutinate rabbit sperm, was also tested for its contraceptive effect in rabbit; this nonagglutinating mAb binds to mouse sperm antigen MSA-63 [19], an intra-acrosomal protein with 60% homology to the human sperm antigen SP-10 [20], a World Health Organization (WHO) candidate contraceptive vaccine antigen [21]. Rabbits were chosen as the model in this study because they are easy to handle, relatively inexpensive, and most frequently used for developing vaginal contraceptives [22]. Several investigations have explored the contraceptive effect in rabbits of polyclonal [23–27] and mAbs [28] against rabbit sperm, but no investigation of the contraceptive effect of sperm-agglutinating mAbs has been reported in rabbit or in any other animal model.

MATERIALS AND METHODS

Materials

Animals. Sexually mature, randomly bred New Zealand White rabbits were used for these experiments. BALB/c female mice, 8–12 wk of age, were used for feeder cells, mAb production, and ascites production. All animal procedures were designed to minimize pain and discomfort, and were approved by Johns Hopkins HAUC.

Semen and sperm collection. Rabbit semen was collected from bucks of proven fertility using a teaser female and an artificial vagina. For rabbit epididymal sperm, the epididymis from a killed male was excised and placed into a Petri dish with Dulbecco's phosphate-buffer saline (DPBS), and epididymal sperm were released by dicing the tissue. Human semen was collected by masturbation and allowed to liquify for 30 min at 37°C.

Production of mAbs

To produce IgM mAbs, a mouse received injections of 10^8 "swim-up" rabbit sperm without adjuvant; half of the sperm were injected intraperitoneally (i.p.) and half injected s.c. at four sites [16]. Seven days after immunization, the animal was killed to obtain the spleen. To produce IgG

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mAbs [17], a mouse was immunized three times i.p. with washed 10^8 rabbit sperm: Day 1 in a 1:1 emulsion with complete Freund's adjuvant (CFA), and Days 15 and 29 in a 1:1 emulsion with incomplete Freund's adjuvant (IFA). The animal was killed on Day 33 for the spleen.

Spleen cells and mouse myeloma cells (P3-NSI/1Ag4.1) were fused for hybridoma production according to a procedure described elsewhere [29]. On Day 10, the hybridomas were screened for sperm agglutination by transferring 30 μl of supernatant from each well to a separate 96-well plate, adding 30 μl of 1:25 diluted rabbit semen in Dulbecco's Modified Eagle's Medium (DMEM; Gibco-BRL, Grand Island, NY), and inspecting for complete, stable agglutination of all rabbit sperm. The positive wells were cloned using either limiting dilution or single-cell picking to achieve monoclonal cell lines.

To produce MARS-M3 ascites, mice were first primed with an i.p. injection 0.5 ml IFA followed 7 days later with an i.p. injection of 10^6 hybridoma cells. Ascitic fluid was pooled, clarified, and heated to 56°C for 30 min to inactivate complement. MARS-M3 was partially purified from the ascitic fluid by polyethylene glycol precipitation (PEG 6000; Fluka Chemical Corp., Hauppauge, NY) [30].

Isotype Determination and Immunoglobulin Quantification by ELISA

Ninety-six-well plates were coated with heavy chain-goat anti-mouse immunoglobulin (Southern Biotechnology Associates, Birmingham, AL, or Vector Laboratories, Burlingame, CA). After being blocked with 3% BSA and 0.05% Tween 20 in PBS, wells were treated sequentially for 1 h with a MARS mAb, with biotinylated goat anti-mouse immunoglobulin (Southern or Vector) of the same heavy chain specificity as the coating antibody, and with horseradish peroxidase (HRPO)-conjugated avidin-biotin complex (Vector). HRPO was detected by the addition of 0.4 mg/ml ortho-phenyl diamine (Sigma Chemical Co., St. Louis, MO) with 0.33 μl/ml 30% hydrogen peroxide in a citrate buffer (0.1 M citric acid/0.2 M Na_2HPO_4), and read on a plate reader at 490-nm wavelength. Subclasses were determined using a commercial isotyping kit (Sigma). Affinity-purified mouse IgM (Zymed, South San Francisco, CA) and protein A-purified MARS-G16 and MARS-G17 were used to calibrate ELISAs to determine MARS-M3, -G16, and -G17 antibody concentrations, respectively, in supernatants.

Indirect Immunofluorescence

Aliquots of 20 μl of ejaculated rabbit semen were treated sequentially for 20 min with either a mAb, a nonspecific mouse immunoglobulin (Zymed), or a biotinylated goat anti-mouse immunoglobulin, and with fluorescein-conjugated avidin (Vector). The unfixed, treated sperm were then pipetted onto a glass slide, sealed under a cover slip, and observed by epifluorescence. To determine whether pretreatment with MARS-G17 blocks binding by G16, protein A-purified G16 was biotinylated with biotin-(long arm)-*N*-hydroxysuccinimide (Vector) according to the manufacturer's protocol and dialyzed against PBS to remove free biotin. An aliquot of rabbit semen was first treated for 20 min with G17 at a final concentration of 2.5 mg/ml while a second aliquot remained untreated before the sequential additions of biotinylated G16 and fluorescein-avidin.

Agglutination and Sperm Immobilization In Vitro

To determine the minimum and maximum sperm agglutination concentrations in vitro, serial twofold dilutions were made in PBS of protein A-purified G16, and G17 and M3 ascites. One part of antibody was mixed with one part diluted semen (10^8 sperm/ml). The mixture was immediately loaded into a 10-chamber slide and, after 5 min, observed in a brightfield microscope for agglutination. The lowest and highest concentrations at which all the sperm were agglutinated were considered the minimum and maximum agglutinating concentrations. The ability of each mAb to agglutinate washed rabbit epididymal sperm and human ejaculated sperm in semen was tested at sperm concentrations of 10^7–10^8 sperm/ml.

Sperm immobilization by complement fixation was tested as above for sperm agglutination but in the presence of 25% rabbit or guinea pig complement. The sperm were then inspected for motility after 30 min, and the monoclonal concentration at which 50% of the sperm were immobilized (SI_{50}) was recorded. Guinea pig complement serum (Sigma), rabbit complement serum (Pel-Freez, Rogers, AR), and freshly collected rabbit serum were used as complement sources; rabbit complement was also tested after being heated either by incubation at 56°C for 30 min or after the addition of 25 mM EDTA.

Immunological Detection of MARS Antigens by SDS-PAGE/Western Blot

Nonylphenoxy polyethoxy ethanol (NP-40) lysates of sperm. Ejaculated rabbit semen was washed three times with 10 ml DPBS and pelleted by centrifugation at 500 × *g* for 8 min. To the final pellet, 1% NP-40 in lysis buffer (50 mM TRIS, 150 mM NaCl, 5 mm EDTA, 1 mg/ml PMSF, single-strength protease inhibitor cocktail (formula given below) was added so that the final sperm concentration was 10^8 sperm/ml. The samples were incubated for 30 min on ice with a second addition of protease inhibitor cocktail after 15 min. Lysates were centrifuged at 14 000 × *g* for 30 min at 4°C, and the lysate supernatants were filtered through 0.22-μm filters.

Protease inhibitor cocktail (500-strength). Protease inhibitor cocktail was prepared by dissolving 0.5 mg/ml leupeptin, 0.25 mg/ml antipain, 0.5 mg/ml aprotinin, 0.25 mg/ml chymostatin (all from Calbiochem-Bering Corp., LaJolla, CA), and 0.5 mg/ml soybean trypsin inhibitor (Sigma) together in distilled, deionized water.

Electrophoresis and Western blotting. The lysates and biotinylated molecular weight standards (Bio-Rad Labs, Richmond, CA) were loaded onto an analytical SDS/PAGE gel under nonreducing conditions (Mini-Protean II; Bio-Rad) [31]. Separated proteins were electrotransferred at 3 mA/cm^2 membrane for 40 min using a semidry transfer blotter (Millipore, Bedford, MA) onto an Immobilon-P membrane (Millipore). Membranes were developed using a chemiluminescent blot detection system (Immun-Lite; Bio-Rad) following the manufacturer's protocol. Nonimmune IgM and IgG (Zymed) were tested as negative controls for primary antibodies in Western blots.

Artificial Insemination (AI) Experiments

To test whether the MARS antibodies have a contraceptive effect, mAbs or controls (PBS or hybridoma medium) were mixed with rabbit semen before artificial insemination. Similarly, two control antibodies were also tested:

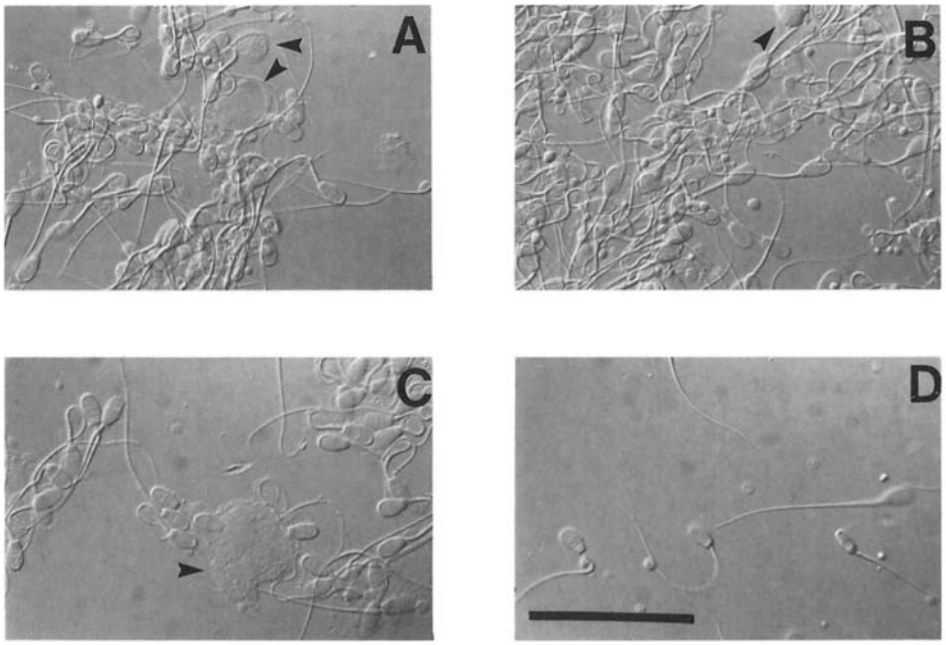

FIG. 1. Nomarski images of live rabbit sperm treated with M3 (**A**), G16 (**B**), G17 (**C**), and nonspecific IgG (**D**). Antibody concentration was 50 μg/ml, and rabbit sperm concentration was 5×10^7. Coagglutination of round cells and debris is visible and indicated by arrows. Bar = 50 μm.

HS-63, a mouse anti-human sperm mAb that blocks in vitro fertilization but does not agglutinate rabbit sperm (Vancouver Biotechnology, Vancouver, BC, Canada), and protein A-purified mouse immunoglobulin from freshly collected nonimmune mouse serum. Females were initially ovulated with intravenous injection of 100 U of hCG (Sigma) into the marginal ear vein within 1 h of insemination. Semen was collected from bucks of proven fertility and used within 1 h of collection. Each hCG-treated female was restrained, and inseminated with a mixture of 1.9 ml test agent and 0.1 ml rabbit semen using a 19-cm cannula attached to a 5-cc syringe. The cannula, made of tygon tubing (3/16” o.d., 1/16” i.d.) and tipped with a bored-out 5/16“(0.795-cm)-diameter silicone-coated ball, was inserted into the vagina to the os of the cervix, where the sample was slowly deposited. The cannula was then slowly removed, and the animal was returned to the cage. The inseminated females were killed by cervical dislocation 8–12 days later, and the number of implants was recorded.

Statistics. Differences in pregnancy and fertilization rates for treated and control animals were evaluated statistically by Fisher's exact tests and by two-tailed paired *t*-tests, respectively. The Wilcoxon rank sum test was used for interstudy comparison of two different treatments.

RESULTS

Sperm-agglutinating mAbs were readily obtained in part because sperm agglutination provides a rapid screening method; wells that demonstrated permanent, complete sperm agglutination were chosen for cloning. The first immunization, a single multiple-site immunization, resulted in a small panel of 7 IgM mAbs [16] including MARS-M3. A second fusion, from a multiple immunization protocol, resulted in many wells positive for agglutination, of which a panel of 4 IgM and 8 IgG$_3$ mAbs including MARS-G16 [17] and MARS-G17 were selected and cloned.

Sperm Agglutination In Vitro

Agglutinated clusters of sperm formed rapidly when these MARS antibodies were mixed with semen (Fig. 1); in contrast, nonspecific mouse IgG (Fig. 1D) and IgM (data not shown) did not cause any detectable agglutination. Although sperm agglutination was evaluated at 5 min, these mAbs agglutinated sperm immediately on mixing, and large agglutinated sperm complexes developed in less than 1 min. The complexes display “mixed agglutination,” in which the mAbs form cross-links head-to-head and tail-to-head, as well as tail-to-tail. The mAbs strongly agglutinated epididymal sperm from both the cauda and caput epididymidis of rabbit. In addition, all three antibodies displayed “pan-semen” activity [1]; i.e., when the mAbs were added to semen, both sperm and round cells coagglutinated, cell surfaces became “fuzzy” or coated with subcellular debris, and the areas between cells became cleared of visible particulates. However, none of these MARS antibodies agglutinated human sperm, and they are thus limited to investigative use in rabbit.

Indirect Immunofluorescence of Rabbit Sperm

Indirect immunofluorescence was performed on unfixed cells, and many sperm remained motile after labeling. Indirect immunofluorescence labeling by M3, G16, and G17 of unfixed ejaculated rabbit sperm (Fig. 2) provided further evidence of the pan-semen characteristics of these antibodies; round cells and debris in semen as well as sperm became fluorescent, indicating that the antigens for these mAbs are present on round cells and debris as well as sperm. Thus these sperm-agglutinating mAbs form cross-links between round cells, particulates, and sperm; they do not simply entrap other cells and debris in the process of cross-linking sperm. Indirect immunofluorescence with M3 (Fig. 2A) showed predominantly patchy labeling of the tail and some on the midpiece, although some sperm demonstrated labeling over the entire surface. Indirect immunofluorescence with nonspecific mouse antibodies did not label sperm, round cells, or visible particulates (Fig. 2B). Indirect immunofluorescence images with MARS-G16 and MARS-G17 mAbs were indistinguishable from those of rabbit sperm labeled with biotinylated MARS-G16 (Fig. 2C), revealing a unique pattern on some sperm in which the entire sperm was labeled except in a narrow “collar” around the

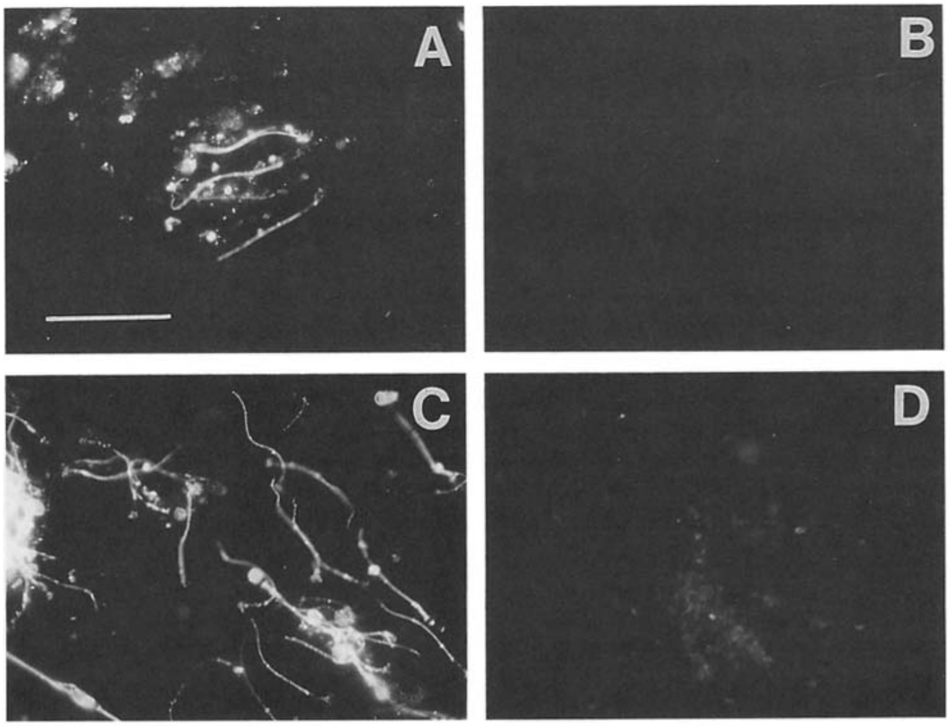

FIG. 2. Indirect immunofluorescence of unfixed rabbit sperm incubated with M3 (**A**) and nonimmune mouse immunoglobulin (**B**). Labeling of round cells and debris by M3 (**A**) and biotinylated G16 (**C**) is visible. **D**) Sperm treated with unlabeled G17 before incubation with biotinylated G16. Note that prior treatment with unlabeled G17 reduces binding by G16, especially on the sperm tails. Bar = 50 μm.

postequatorial region. Even though the antigens for G16 and G17 are ubiquitous on the round cells and particulates in semen as well as all other surfaces of the sperm, for some reason the antigens for these mAbs are missing or obstructed in this postequatorial collar region. When sperm were treated with unlabeled G17 before biotinylated G16 was added, labeling by G16 was markedly reduced on sperm as well as round cells, and especially reduced on sperm tails, suggesting that G17 binds either to the same epitope or to a nearby epitope that sterically hinders simultaneous binding of both G17 and G16 (Fig. 2D).

Relationship of mAb Concentration to Agglutination

Minimum mAb concentration for complete sperm agglutination, $[\mathrm{Agg}]_{\mathrm{min}}$, was 1 μg/ml for M3, 5 μg/ml for G16, and 6 μg/ml for G17 (Fig. 3A). In addition, each mAb had a maximum concentration, $[\mathrm{Agg}]_{\mathrm{max}}$, above which nonagglutinated sperm became visible. The appearance of free sperm at both high and low antibody concentrations is analogous to the formation of immune complexes [32] that form only within a limited range of antibody concentrations: when the antibody concentration is in large excess with respect to the antigen concentration, cross-linking between antigens decreases. The $[\mathrm{Agg}]_{\mathrm{max}}$ was 3200 μg/ml for M3, 600 μg/ml for G16, and 1200 μg/ml for G17 (Fig. 3A). As expected, because of its polyvalency, M3 maintained complete agglutination over the broadest concentration range.

Contraceptive Effect of mAbs In Vivo

Figure 3B shows the contraceptive effects of MARS antibodies. Of the four MARS antibodies tested, G16 was the most effective in blocking conception: 150 μg of G16 reduced the pregnancy rate by 69% ($p = 0.001$) and the number of fetuses/animal (fertility) by 88% ($p = 0.0001$) as compared to the control. The dose-response relationship for contraceptive efficacy was investigated at three additional doses with protein A-purified G16 (Fig. 3B); 2 μg did not prevent conception, 20 μg reduced the number of fetuses per animal by 29% (a decrease too small to be statistically significant), and 2000 μg reduced the pregnancy rate by 78% ($p < 0.005$) and the number of fetuses per animal by 95% ($p = 0.0005$) as compared to the controls. M3 (400 μg) and G17 (180 μg) reduced fertility by 68% ($p < 0.05$) and 47% ($p < 0.05$), respectively, as compared to controls (Fig. 3B). M3, G17, and G16 at 150 μg and 2 mg were all statistically different from each other.

By comparison, 2 mg of nonimmune, protein A-purified mouse immunoglobulins did not reduce fertility, evidence that the contraceptive effects observed were due to the specificity of the mAbs. In addition, 2 mg of HS-63, a mouse mAb that binds to an intra-acrosomal antigen, did not prevent conception.

Complement-Mediated Sperm Immobilization

To investigate whether complement-mediated immobilization might contribute to the contraceptive effects of these pan-semen mAbs, rabbit sperm were treated with these mAbs in the presence of complement. In the presence of guinea pig complement, M3, G16, and G17 immobilized rabbit sperm with an SI_{50} of 0.3 μg/ml, 1.3 μg/ml, and 3.6 μg/ml, respectively. In marked contrast, rabbit complement not only failed to immobilize the sperm; it induced "disagglutination" of sperm, allowing sperm to swim free of the agglutinated complexes; after 30 min, rabbit complement freed most sperm from agglutinated complexes, with M3 demonstrating longer-lasting agglutination than the IgGs. When rabbit complement was added only after complete agglutination had been induced by a MARS antibody, the complement could still rapidly disagglutinate many sperm. Rabbit complement that was heated for 30 min at 56°C, or to which EDTA was added to chelate free Ca^{2+} as required for complement activation, almost completely lost its ability to disagglutinate sperm.

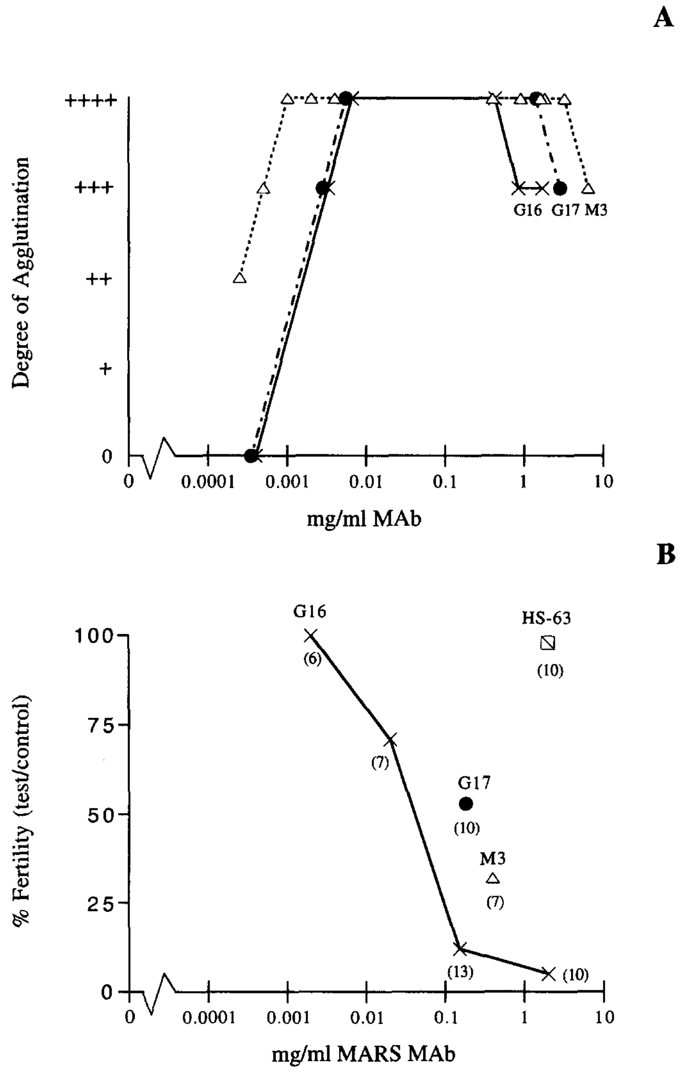

FIG. 3. A) Degree of agglutination of rabbit sperm in vitro as a function of antibody concentration for M3, G16, and G17. Sperm agglutination was determined using serial twofold dilutions of the mAbs with a final concentration of rabbit sperm of 5×10^7/ml. Vertical axis: ++++, complete agglutination or no visible free, individual sperm; +++, strong agglutination with an occasional free sperm; ++, significant sperm agglutination but many free sperm; +, sperm agglutination apparent but mostly free sperm; 0, no sperm agglutination. B) Contraceptive efficacy of M3 (triangle), G17 (filled circle), HS-63 (hatched square), and G16 (cross), tested at several antibody concentrations. Contraceptive efficacy was tested by mixing 1.9 ml of antibody at the concentrations indicated with 0.1 ml semen before the inseminating sample was deposited at the cervices in the rabbit. Contraceptive efficacy as indicated by % Fertility (test/control) is the average number of fetuses per animal of the test group expressed as a percentage of the average number of fetuses per animal in the control group. The numbers of animals tested in a group are in parentheses.

SDS-PAGE/Western Blots of Sperm Lysates

For a sensitive assay to help determine whether MARS antigens were related to other reported rabbit sperm antigens, SDS PAGE/Western blots were performed on sperm lysates of ejaculated rabbit sperm and human sperm. With a 1% NP-40 lysate of rabbit sperm on a 13% acrylamide gel, both G16 (Fig. 4A, lane 2) and G17 (Fig. 4A, lane 3) labeled a major band of 13 kDa apparent molecular mass whereas nonimmune mouse IgG failed to label any protein bands (Fig. 4A, lane 1). With a 1% NP-40 lysate of rabbit sperm on a 10% acrylamide gel, M3 labeled a single band of 52 kDa (Fig. 4B, lane 2) whereas nonimmune mouse IgM failed to label any protein bands (Fig. 4B, lane 1).

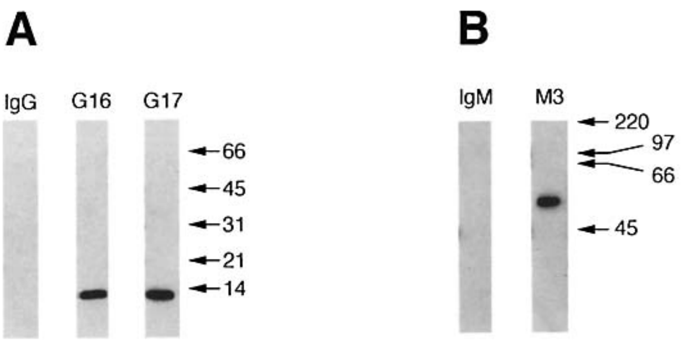

FIG. 4. Analysis of MARS antigens in rabbit sperm lysates by SDS-PAGE/Western blots. A) Lysate was electrophoresed on 13% acrylamide gel. Lane 1 was probed with a nonimmune mouse IgG, lane 2 was probed with G16, and lane 3 was probed with G17. B) Lysate was electrophoresed on 10% acrylamide gel. Lane 1 was probed with nonimmune mouse IgM, and lane 2 was probed with M3. Arrows indicate the mobility of biotinylated molecular weight standards (× 10^{-3}).

DISCUSSION

Sperm-Agglutinating Antibodies and Their Antigens

The rabbit antigens reported here do not appear to be related to previously reported sperm antigens on the basis of apparent molecular size and the unique indirect immunofluorescence pattern of MARS-G16 and MARS-G17 on unfixed sperm [33, 34]; however, similar antigenic patterns have been reported on fixed sperm [27, 35]. One of these, RSA-1 [35–37], the best-characterized rabbit sperm antigen, is a sialoglycoprotein with a molecular-mass isoform of 13 kDa, similar to the molecular mass of antigen bound by G16 and by G17. But this 13-kDa isoform of RSA-1 is obtained only from the testis; on ejaculated sperm, the major RSA-1 band has a molecular mass of 84 kDa [37]. Thus, G16 and G17 may bind some antigen other than RSA-1. The relationship between antigens for G16 and G17 is uncertain. Preliminary data using Western blots and ELISA have demonstrated that the G17 antigen, but not the G16 antigen, is present in rabbit seminal plasma, suggesting that each mAb may bind to a different epitope. However, the two epitopes are either overlapping or in close enough proximity on rabbit sperm so that binding by G17 sterically blocks binding by G16.

The antigens bound by the MARS mAbs are present on round cells and seminal debris as well as sperm, resulting in coagglutination. Antibodies against such antigens have previously been described as pan-semen antibodies [1].

Contraceptive Efficacy

The contraceptive effect of the MARS mAbs in the rabbit reported here is consistent with clinical evidence in humans that the presence of sperm-agglutinating antibodies in semen reduces fertility. The contraceptive efficacy achieved by the 150-μg and 2-mg doses of MARS-G16 are comparable to that achieved in previous tests in rabbits with polyclonal [23–27] and monoclonal antibodies [28]. Although sperm-agglutinating mAbs were contraceptively effective, even a dose of 2 mg G16 did not provide 100% efficacy. This residual fertility suggests that some process in the upper reproductive tract of the rabbit frees at least a small number of sperm from agglutination, even though in semen, once all sperm became agglutinated none were ever observed to become free again.

A rabbit ejaculate delivers far more fertilizing doses than does a human ejaculate, and thus the rabbit provides a rigorous test animal for vaginal contraceptive agents. For example, when delivered paracervically, as in the present investigation, as few as 5×10^4 rabbit sperm produced maximum fertility [38], and 2×10^4 rabbit sperm is estimated to be the dose for 50% maximum fertility or the FD_{50} (unpublished results). The inseminating dose (0.1 ml semen) used in this investigation delivered approximately $5–10 \times 10^7$ sperm, or 2500–5000 FD_{50}. Therefore, to achieve a 50% reduction in fertility in these tests, the mAb must reduce the number of effective sperm by at least 2500-fold. By comparison, a human ejaculate probably contains no more than 1 FD_{50} [39]. Thus, in terms of fertilizing doses, the dose of rabbit semen used in this study is on the order of several thousand human ejaculates. These comparisons of sperm dose suggest that in humans, an antibody no more effective than MARS-G16 in reducing the fertilizing capacity of semen might be essentially 100% contraceptive.

Contraceptive Mechanisms

Monoclonal antibodies provide an opportunity for elucidating how antibodies against sperm block conception. For example, the sperm-agglutinating antibodies used in this study did not immobilize (kill) sperm, but they did eliminate forward motility. Thus they are likely to prevent conception, at least in part, by inhibiting all processes that depend on forward motility by sperm, i.e., cervical mucus penetration [9–13, 40] and possibly sperm-egg interactions [13–15].

Our observations do not establish whether agglutination is the only mechanism by which MARS antibodies prevent conception, but the dose-response relationship for G16, the most effective contraceptive mAb tested in this investigation, shows a reasonable correlation between the degree of sperm agglutination and contraceptive efficacy. Considering that fluids in the female tract must partially dilute the antibody, the results in Figure 3 indicate that G16 was highly effective contraceptively only in the dose range in which it fully agglutinates sperm. In contrast, 2 mg of HS-63 mixed into semen did not reduce fertility even though this antibody blocks in vitro fertilization [19]. This antibody binds to an intra-acrosomal antigen that becomes well exposed only after the acrosome reaction. Its failure to prevent conception implies that the 2-mg dose added to the semen was insufficient to insure that a contraceptively effective dose reached the oviducts. These findings suggest that anti-sperm mAbs used in barrier contraceptives must bind to sperm in semen in the vagina in order to be contraceptively effective.

Complement has previously been shown to immobilize antibody-coated sperm, and sperm-immobilization by complement has been proposed as an effector mechanism for immune infertility [14, 41]. However, in this study, the presence of rabbit complement resulted in the release of free, motile sperm from agglutinated complexes of sperm rather than in the immobilization of sperm. The disagglutination of sperm is possibly related to complement-mediated solubilization of immune complexes [42]. In this process, the C3b component of the complement reported becomes incorporated into the immune complex and dissociates primary antigen-antibody bonds [43], which, in turn, cause the antibodies to become functionally univalent [44]. Our inability, using indirect immunofluorescence, to detect any loss of antibody from the sperm surface after complement-mediated disagglutination suggests that disagglutination results from the antibody's becoming univalent rather than lost from the sperm surface. Our results with rabbit complement suggest that the residual fertility we observed with the MARS antibodies obtained from mouse may have been caused by disagglutination of motile, fertile sperm by complement activity in the upper reproductive tract of the female rabbit. Whether this complement-mediated phenomenon will reduce the contraceptive effectiveness of human anti-sperm mAbs applied vaginally is unknown.

Conclusions

The MARS mAbs used in this study were effective contraceptive agents when mixed into semen and delivered vaginally in rabbits. Since the rabbits were inseminated with several thousand fertilizing doses (2500–5000 FD_{50}), and since a human ejaculate probably delivers no more than 1 FD_{50}, a sperm-agglutinating mAb might prove to be significantly more effective in humans.

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